The coming of age of immuno-oncology: IL-2 therapy sets the stage

Immuno-oncology- An introduction:
Immuno-oncology represents a revolutionary and innovative approach to cancer treatment that seeks to activate the body's own immune system to combat tumors. Although the emergence of immunotherapy as a first line of treatment for cancer patients is relatively recent, the idea that the immune system can be provoked to respond to tumors dates back to the late 19th century. William Coley, an American cancer researcher, observed that patients who developed post-operative infections displayed better tumor regressions than those without infections. Coley further reported that injection of bacterial toxins (killed cultures of *Streptococcus pyogenes* and *Serratia marcescens*) into the tumor sites of sarcoma patients, led to complete response rates in 10-15% of the patient population. Coley theorized that the high rates of tumor regression associated with infections, whether incidental or by design, was due to the body's ability to harness the potential of the immune system to mount a response against the tumors (Mellman et al., 2011). Coley's pioneering work laid the foundation for the field of immuno-oncology, leading to subsequent research efforts that have shed light on the inherent surveillance mechanisms of the immune system and its ability to recognize and reject tumors (Eggermont, 2012; Hoos and Britten, 2012).

One of the goals of immuno-oncology is to understand the molecular players contributing to immune system surveillance and the mechanisms by which cancer cells evade detection. Studies that examine interactions between the immune system and tumors have revealed that cancer-bearing hosts can activate both the innate and adaptive arms of the immune system to mount an effective anti-tumor immune response (Chow et al., 2012). Cytokines are an essential class of molecular messengers that influence both the innate and adaptive immune responses and are produced in response to several immunogenic stimuli including tumor antigens. Given their essential roles in the etiology of cancer, significant efforts have focused on manipulating the cytokine balance in cancer therapy. Interleukin-2 (IL-2) is one such cytokine that has been widely studied in the pathogenesis of tumors as well as in cancer immunotherapy (Dranoff, 2004; Finn, 2008; Lee and Margolin, 2011).

IL-2: A paradigm for cytokine signaling
Discovered in 1976, IL-2 was initially termed "T cell growth factor", based on its ability to stimulate the growth of T cells. It is now known to be a member of a family of cytokines that include IL-4, IL-7, IL-9, IL-15, and IL-21, all of which play an important role in the maintenance of T cell populations (Morgan et al., 1976). In addition to functioning as a mitogen for T cells, IL-2 was subsequently discovered to perform multifaceted functions in both the innate and adaptive immune responses by sustaining the growth and expansion of other lymphocyte subtypes, natural killer (NK) cells and B cells, promoting differentiation of T cells into effector T cells and memory T cells and augmenting the cytotoxicity of monocytes (Buchbinder and McDermott, 2014; Malek and Castro, 2010). Paradoxically,
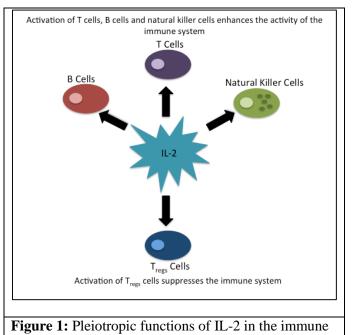
IL-2 is also important for the development and expansion of T regulatory cells (T$_{regs}$) that serve to dampen effector T cell responses. IL-2, thus, plays a critical role in regulating the balance between immunity and tolerance (Figure 1) (Malek and Castro, 2010; Sakaguchi et al., 2008).

IL-2 signaling is potentiated through its cognate IL-2 receptor (IL-2R), which belongs to the family of Type 1 cytokine receptors. IL-2R is composed of three subunits, IL-2Rα (CD25), IL-2Rβ (CD122) and the common IL-2Rγ (γc) subunit that is shared by all other family members. While all three subunits are required for high-affinity binding of the cytokine, IL-2 forms an intermediate-affinity complex with the β and γ subunits alone that is fully capable of transducing a signaling reaction. The formation of the IL-2-IL-2R complex leads to activation of the tyrosine kinases Janus kinase 1 (Jak1) and Jak3, which are associated with IL-2R β and, γ respectively. Activation of Jak1 and Jak3 further triggers downstream cell signaling pathways including the Janus Kinase and Signal Transducer and Activator of Transcription (JAK/STAT) pathway, mitogen-activated protein kinase (MAPK) pathway, and the phosphatidylinositol 3-kinase (PI3K) pathway (Skrombolas and Frelinger, 2014).

Expression levels of IL-2 and IL-2R are highly regulated in different immune cells. IL-2 is present at low levels in the normal serum and exerts its effects over short distances through autocrine or paracrine mechanisms. Although primarily secreted by activated CD4 T cells, IL-2 production by CD8 T cells, NK cells, as well as activated dendritic cells has also been reported. Distinct immune cells also express varying amounts and combinations of the IL-2R subunits. Resting naïve T cells express very low levels of IL-2Rs. However, activated CD4 and CD8 T cells express high levels of IL-2Rs, particularly the α subunit. The α subunit by itself does not mediate any signaling activity but aids in binding to IL-2, localizes it to the cell surface and further induces a conformational change in IL-2 allowing it to bind with the βγ subunits. In addition to T cells, the β and γ subunits of IL-2R

Figure 1: Pleiotropic functions of IL-2 in the immune system

are highly expressed on NK cells and memory CD8 cells as well. (Liao et al., 2013; Wang et al., 2009) Thus, the different expression patterns of both IL-2 and IL-2R by immune cells dictate the potency and functions of IL-2-mediated signaling *in vivo*. Given its important role in in the immune system, particularly in the expansion and maintenance the T cell population, IL-2 was one of the first cytokines tested in cancer patients for therapeutic gain.
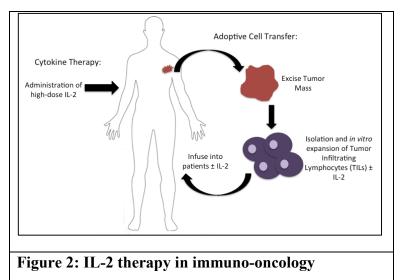
IL-2 therapy in human and veterinary cancers:

Steven A. Rosenberg is an eminent surgeon and cancer researcher who has pioneered the use of IL-2 as an immunotherapy drug in certain forms of metastatic cancer. In a seminal study published by Rosenberg and colleagues, high-dose IL-2 was administered to patients with melanoma and renal cancers. In what is considered a breakthrough study for the field on immuno-oncology, 23% of renal cell cancer patients and 29% of melanoma patients reported having significant cancer regressions. This was the first demonstration of the benefits of manipulating the immune system for cancer therapy. The success of this study led to a surge in the number of trials with IL-2 treatment in metastatic patients, eventually leading to FDA approval of high-dose IL-2 administration in metastatic renal cancer patients followed by its approval for metastatic melanoma. In both these tumor indications, IL-2 treatment is associated with tumor regression in about 20% of patients, with complete responses in about half of the responders. Importantly, a fraction of the patients remain tumor free beyond 10 years of treatment, showing remarkable durability, which we now know is a hallmark of immunotherapy responses in cancer. It is however important to note that other than in a few patients with advanced non-Hodgkins lymphoma, most other tumor types were largely refractory to IL-2 therapy (Eklund and Kuzel, 2004; Rosenberg, 2014; Rosenberg et al., 1994; Rosenberg et al., 1998).

Despite the overwhelming success rate in a small minority of patients, IL-2 therapy has been fraught with serious side effects, limiting its extensive use in cancer treatment. One deleterious side effects of IL-2 therapy occurs as a result of widespread stimulation of different immune cells, leading to highly elevated levels of different cytokines, termed "cytokine storm". The primary manifestations of a cytokine storm are fever, swelling, and fatigue, which can be fatal in some cases. Vascular leak syndrome (VLS), another common side effect associated with IL-2 therapy is characterized by increased vascular

Figure 2: IL-2 therapy in immuno-oncology

permeability, often leading to interstitial edema and organ failure. While titrating the dosage of IL-2 has been explored, lower doses have generally proven to be less effective clinically. IL-2 also has a short half-life in serum, necessitating large and multiple doses of IL-2 in patients, further compounding toxicity concerns. While the basic premise of IL-2-based immune-therapy is to tap into IL-2's ability to activate T cells, the aforementioned dual role of IL-2, functioning both as an activator and suppressor of the immune system, further poses a challenge to its efficacy in cancer immunotherapy. The activation of T_{regs} in circulation and the tumor microenvironment has an overall suppressive role on the immune system, limiting the efficacy of IL-2 therapy (Mekhail et al., 2000; Rosenberg, 2014).

Several strategies are being tested to overcome these hurdles associated with IL-2 administration in patients. To mitigate the systemic toxicity that patients on IL-2 therapy often endure, scientists are exploring mechanisms to deliver IL-2 directly to the tumor site. Through local delivery, the aim is to diminish many of the systemic side effects including VLS that are otherwise quite common. Another strategy to improve the efficacy of IL-2 therapy has been to increase the serum half-life of IL-2, to alleviate the need for administrating high doses of the cytokine. By fusing IL-2 to human albumin, the half-life of IL-2 was shown to increase from 19 minutes to 7.75 hours in mice. Importantly, this version of IL-2 maintained its properties of tumor reduction in mouse kidney tumor and melanoma models (Eklund and Kuzel, 2004; Skrombolas and Frelinger, 2014).

IL-2 has also been tested in adoptive cell transfer (ACT), a technique that involves isolation of tumor infiltrating lymphocytes (TILs) from surgically excised tumors, expanding the cells *in vitro* and reintroducing them back into the patient (Mellman et al., 2011). The first successful demonstration of ACT was by Rosenberg and colleagues who observed tumor regression in about 60% of patients with metastatic melanomas treated with *in vitro*-expanded TILs in concert with IL-2 (Rosenberg et al., 1988). Several different adaptations of this study have since been tested in different forms of cancers with varying results. In human renal cell cancers, TILs were expanded in the presence of IL-2 and the potency of the activated TILs to regress tumors was tested in the presence and absence of IL-2 administration. The cytokine is administered to further support the proliferation and potency of the T cells, but IL-2-associated toxicity has limited the implementation of the technique. Several trials are ongoing to investigate toxicity profiles and optimal conditions associated with ACT with an aim to yield long-lasting responses in different tumor types with minimal side effects (June, 2007; Rosenberg et al., 2008). Given the plethora of ideas to improve the overall success of IL-2 in cancer immunotherapy, there is a pressing need for further research to bring these ideas to fruition.

Several studies are also underway to examine the roles of IL-2 therapy in veterinary cancer. Recombinant human (rhIL-2) has been used with great success to treat bovine ocular squamous cell carcinomas (Den Otter et al., 1995). In this study, 67% of the sample size tested exhibited complete regression 20 months post-therapy. A pilot study conducted in dogs with mast cell tumors reported that local application of human rhIL-2 to dogs exerted a strong therapeutic response with minimal side effects. While the data from the pilot study are promising, a larger study is warranted to further prove the efficacy of IL-2 therapy in mast cell treatment for canines (Ziekman et al., 2013). A recombinant virus expressing feline IL-2 were explored as a form of adjuvant immunotherapy in cats with feline injection-site sarcomas. The treatment group displayed a longer time to relapse and IL-2 treatment proved safe and efficacious (Jas et al., 2015). Oncept IL-2 that expresses feline IL-2 through a recombinant canarypox virus is the first immunological veterinary drug approved in Europe for treatment of cats with fibrosarcoma. Given the commonalities shared between human and veterinary tumors including histological appearance, molecular genetics and response to therapies, studies in animal models such as canines and felines have provided valuable perspectives to the field of cancer research and merit further research to propel the field (Bergman, 2014).

Immuno-oncology today:

The field of immuno-oncology has greatly advanced since the initial characterization and approval of IL-2 therapy for cancer treatment. Use of antibodies that block the activity of the immune checkpoint, programmed cell death protein 1 (PD1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) as therapies has demonstrated remarkable success in patients with metastatic melanoma and lung cancer. Initial activity is also being demonstrated in metastatic renal cancer patients as well as in other cancer indications. In contrast to IL-2, these agents provide a more selective tool to stimulate anti-tumor T-cell activity. Both agents specifically remove the brakes on cytotoxic T cells, in contrast to a general activation of the immune system (Dolan and Gupta, 2014; Hoos and Britten, 2012). The promising results with the newer generation immuno-oncology drugs had led to renewed enthusiasm in the field and immunotherapy is now firmly positioned in mainstream clinical oncology. Immuno-oncology continues to revolutionize treatment options for patients with metastatic cancers across many different tumor types. As always, further advances in the field will require continual generation of reagents and model systems, identification of biomarkers and productive collaborations between basic scientists and clinicians.

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